



The pertussis hypothesis: *Bordetella pertussis* colonization in the pathogenesis of Alzheimer's disease

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ABSTRACT

While a number of endogenous risk factors including age and genetics are established for Alzheimer's disease (AD), identification of acquired, potentially preventable or treatable causes, remains limited. In this paper, we review three epidemiologic case studies and present extensive biologic, immunologic and anatomic evidence to support a novel hypothesis that *Bordetella pertussis* (BP), the bacterium better known to cause whooping cough, is an important potential cause of AD. Cross-cultural documentation of nasopharyngeal subclinical BP colonization reflecting BP-specific mucosal immunodeficiency, proximate anatomy of intranasal mucosal surfaces to central nervous system (CNS) olfactory pathways, and mechanisms by which BP and BP toxin account for all hallmark pathology of AD are reviewed, substantiating biologic plausibility. Notably, respiratory BP infection and BP toxin secreted from subclinical BP colonization can account for the initiation and accumulation of amyloid β plaques and tau tangles. Additional mechanisms consistent with the immunobiologic effects of subclinical BP colonization include microglial activation and inflammation, atrophy and neurodegeneration, excitotoxicity, distinctive anatomic distribution and sequential spread of disease, impaired glucose utilization, and other characteristic CNS pathology of AD. We conclude by assessing the evidence for causation against the Bradford Hill criteria, and advocate for further investigation into the potential role of BP in the etiology of AD.

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1. Introduction

First described in 1907 (Alzheimer, 1907), Alzheimer's disease (AD) is a neurodegenerative disorder characterized by slowly progressive cognitive and behavioral impairment in those with

Abbreviations: AD, Alzheimer's disease; BP, *Bordetella pertussis*; CNS, central nervous system; A β , amyloid β ; NFT, neurofibrillary tangle; APOE, apolipoprotein E; HSV-1, herpes simplex virus-1; A β PP, A β precursor protein; BACE-1, β -site amyloid precursor protein-cleaving enzyme; US, United States; wPV, whole cell pertussis vaccine; aPV, acellular pertussis vaccine; SCBP, subclinical *Bordetella pertussis* colonization; DPT, diphtheria pertussis and tetanus vaccine; PCR, polymerase chain reaction; CDC, Centers for Disease Control and Prevention; CVO, circumventricular organ; BBB, blood-brain barrier; kDa, kilodalton; CSF, cerebrospinal fluid; cAMP, cyclic adenosine monophosphate; PKA, protein kinase A; Th1, T helper 1; Th17, T helper 17; mRNA, messenger ribonucleic acid; IL-1 β , interleukin 1 β ; TNF- α , tumor necrosis factor- α ; IL-6, interleukin 6; GABA $_B$, gamma-aminobutyric acid B; BDNF, brain-derived neurotrophic factor; CGRP, calcitonin gene-related peptide; sAPP α , soluble amyloid precursor protein α ; GSK3 β , glycogen synthase kinase 3 β ; ATP, adenosine triphosphate; NAD $^+$, nicotinamide adenine dinucleotide; NADPH, nicotinamide adenine dinucleotide phosphate; GLUT4, glucose transporter 4; GRK2, G-protein-coupled receptor kinase 2; GA, Golgi apparatus; ER, endoplasmic reticulum; PS, presenilin; MS, multiple sclerosis.

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intracellular cerebral neurofibrillary tangles (NFTs) composed of abnormal tau protein, and extracellular plaques composed of amyloid- β (A β) peptides (Hyman et al., 2012; McKhann et al., 2011). Concordance rates for AD in monozygotic twins vary from 21 to 83% (Gatz et al., 2006; Breitner et al., 1995; R  ih   et al., 1996), the wide range in part related to differences in study design and acquired risk factors (Gatz et al., 2006). Notably, when a genome-wide analysis of genetic loci associated with Alzheimer's disease was unable to expand the power of AD prediction models beyond age, sex and apolipoprotein E (APOE) status alone (Seshadri et al., 2010), a call was raised to shift the search for causes of AD "back to the environment" (Pedersen, 2010). In this respect, a role for infection in the pathogenesis of AD has been previously suggested (Honjo et al., 2009; MacDonald, 1988), and in a recent editorial, 33 AD researchers and clinicians "propose that further research on the role of infectious agents in AD causation... is justified" (Itzhaki et al., 2016).

Several neurotropic infections have been associated with AD, including *Chlamydia pneumoniae*, *Borrelia burgdorferi*, and *Toxoplasma gondii*, though not all investigators confirm these associations (Hammond et al., 2010; Nicolson, 2008; Kusbeci et al., 2011). It is plausible that chronic infections, particularly neurotropic infections, contribute to AD. For example, CNS Herpes Simplex Virus-1 (HSV-1) increases the risk of AD in carriers of the

APOE ϵ 4 allele (Itzhaki and Wozniak, 2008), and mouse brain cells infected with HSV-1 show increased levels of the A β precursor protein (A β PP) cleaving enzyme BACE-1 (β -site amyloid precursor protein-cleaving enzyme) and increased A β deposits (Wozniak et al., 2007). Still other investigations have demonstrated that A β has broad-spectrum antimicrobial properties, raising the possibility that this hallmark of AD is an evolutionary response to bacterial exposure (Soscia et al., 2010). *Corynebacterium diphtheriae*, which infects the human nasopharynx, has also been proposed to cause AD via the passage of diphtheria toxin from olfactory epithelium into the CNS (Merril, 2012), building on previous work suggesting that olfactory pathways may be conduits for microbes and toxins in the etiology of AD (Mann et al., 1988; Ferreyra-Moyano and Barragan, 1989).

Identified in 1906 (Bordet and Gengou, 1906), *Bordetella pertussis* (BP) is a Gram-negative bacterium that secretes biologically active toxins and causes whooping cough (acute clinical BP). Both subclinical and symptomatic BP infections persist in highly vaccinated populations (Ward et al., 2005; Zhang et al., 2014; de Melker et al., 2006; Hallander et al., 2011; van Boven et al., 2000), with escalation of reported whooping cough cases in the past two decades in the United States (US) (<http://www.cdc.gov/pertussis/surv-reporting/cases-by-year.html>) and many other industrialized nations (Chiappini et al., 2013). In 2012, it was noted that “the prevalence of *B. pertussis* infection is high in [US] adults and increasing at a significant rate, especially in individuals more than 65 years of age” (Weston et al., 2012). In an American study of individuals over age 65, BP serology elevations consistent with infection were detected in 10% of subjects over a three year period, and one-half to one-third were asymptomatic (Hodder et al., 2000). Multiple linear regression analysis suggests that between 2006 and 2010 there were 465,000 cases of pertussis infection in US adults age 65 and older, though only 6369 cases were actually reported (Masseria and Krishnarajah, 2015). In short, *Bordetella pertussis* insidiously infects large numbers of adults, including the elderly, and pertussis rates have been rising in recent decades.

Multiple factors are held to play a role in rising US BP rates, including a reduction in vaccine efficacy when the US switched from whole cell pertussis vaccines (wPV) to acellular pertussis vaccines (aPV) in the late-1990s due to wPV safety concerns (Winter et al., 2014). Acellular pertussis vaccines generally contain three or four purified, inactivated components of BP, compared to the broad antigenic BP profile of wPV prepared from heat or chemically inactivated whole bacteria, and it is generally agreed that the transition from wPV to aPV led to less durable immunity (Winter et al., 2014). In addition, aPV is unable to protect against BP nasopharyngeal colonization as demonstrated by Warfel et al. in a non-human primate model, and colonized baboons are able to transmit BP infections to naive baboons in close proximity (Warfel et al., 2014). As such, the authors concluded: “that aP [vaccines]... fails to prevent colonization or transmission provides a plausible explanation for the [US] resurgence of pertussis.” Complementing these data, recent phylogenetic analysis and mathematical modeling studies by Althouse and Scarpino indicate that while “waning immunity plays a role in the epidemiology of pertussis”, “asymptomatic transmission is the most parsimonious explanation for many of the observations surrounding the resurgence of *B. pertussis* in the US” (Althouse and Scarpino, 2015).

Subclinical BP colonization (SCBPC) infections are here defined as asymptomatic or mild infections (e.g. transient cough or rhinorrhea) that elicit minimal or no BP-directed host immunity, thereby allowing unopposed BP toxin activity in a host. To illustrate the relationship between BP infection and AD, we first apply our hypothesis to three environmental observations. Evidence of anatomic and

biological plausibility follow in the third section, and immunobiologic mechanisms are discussed in the fourth section of this review.

2. Epidemiologic case studies

2.1. Case 1: the concurrent age-adjusted rise of AD and BP in the US

Increasing death from AD (Xu et al., 2010) and age-adjusted risk for AD (Akushevich et al., 2013) in the US since the early 1990s coincide with the commensurate upsurge in BP (Clark, 2014), the only vaccine-preventable disease in the US increasing in incidence in recent decades (Brooks and Clover, 2006). While limited to clinically identified AD cases, and though part of the overall rise in AD burden in recent decades is due to increased longevity and a diagnostic shift from diseases with improved survival, we propose, given the totality of evidence presented below, that the age-adjusted rise in AD is a true independent increase, and may be substantially due to escalating BP infection rates.

Others have proposed that systemic inflammatory disease may drive neurodegeneration in AD (Perry et al., 2007). We suggest that systemic inflammatory diseases may contribute to the progression of AD without being sufficient cause for AD alone. In fact, many common diseases with an inflammatory component, including stroke, myocardial infarction, colon cancer, breast cancer, and rheumatoid arthritis (Akushevich et al., 2013), have decreased in age-adjusted incidence and mortality as AD incidence has risen. Additional potential sources of systemic inflammation, such as smoking (CDC, 2011) and periodontal disease (Hugoson et al., 2008), have also dropped over the same period. In summary, we submit that generalized inflammation alone is insufficient to explain the increase in AD in recent decades, distinguishing BP as a specific potential cause that has risen with AD during the same period.

2.2. Case 2: vaccination and reduced AD risk

Diphtheria and tetanus vaccines typically, though not exclusively, are co-administered with BP antigens. In a cohort of 3682 non-demented Canadians 65 years of age and older, self-reported vaccination for diphtheria or tetanus, “considered together, as they are usually given together in vaccination programs,” correlated with a statistically significant 60% (95% CI 35–75%) lower risk for the development of AD over five years during the 1990s (Verreault et al., 2001). No significant association was found between vaccination for influenza and AD, making it unlikely that a boosted nonspecific immune or vaccine response alone is AD-protective. The model adjusted for age, sex, education, alcohol consumption, family history of dementia, measures of activities of daily living, antecedents of chronic diseases, and perceived health status. The cohort percentage that received BP vaccination bundled with diphtheria or tetanus vaccination was not reported, however it is likely that a high proportion had simultaneously received pertussis vaccination because: (1) combination diphtheria, pertussis and tetanus vaccines (DPT), pioneered in Canada, were readily adopted after introduction in 1943 (Varughese, 1985), (2) DPT administration has been routine for school vaccination programs in Canada since 1948 (Verreault et al., 2001), and (3) currently, 9 of 11 available preparations of tetanus toxoid-containing vaccines in Canada are combined with BP antigens (Canadian Immunization Guide, 2016).

A second independent Canadian study of 694 non-demented individuals age 65 and over observed for the onset of AD corroborated the same association between vaccination and reduced AD risk. In this study, a history of vaccination for tetanus or diphtheria, and thus likely BP, was associated with a significantly reduced risk

for diagnosis with AD over five years (RR 0.4, 95% CI: 0.17–0.96) (Tyas et al., 2001).

2.3. Case 3: AD in Nigerians and African Americans

During the mid-1990s, a total of 4606 non-demented residents of Ibadan, Nigeria, and African Americans in Indiana, US, all greater than 65 years of age, were followed for AD onset with standardized and identical screening, sampling, and diagnostic methods (Hendrie et al., 2001). Bias from cohort attrition, mainly due to death, was mitigated by adjustment for mortality. Minor variation in $\epsilon 4$ allele-AD association and differences in vascular disease between groups might have accounted for some risk variance. Participation refusal rates were low in both populations, and few differences were detected between subjects who completed and dropped out of the study. In sum, detection, attrition, and reporting bias were not likely large sources of outcome variation. The age-standardized mortality-adjusted annual incidence of AD was a statistically significant 54% lower among Nigerians (1.15%, 95% CI 0.96–1.35%) than African Americans (2.52%, 95% CI 1.40–3.64%) after only five years of observation (Hendrie et al., 2001). In a previous report from the same investigators, age-adjusted AD prevalence rates were 1.41% in the Nigerian cohort and 6.42% in the African American cohort (Ogunniyi et al., 2000).

To account for the US-Nigerian AD risk differential, we submit that variable rates of subclinical *Bordetella pertussis* colonization (SCBPC) in the two populations played an important role. In the WHO-defined African region, initial BP vaccination series coverage was 5% in 1980, 52% in 2000 and 72% in 2012, when US rates were 96%, 94% and 96% respectively (WHO, 2014). We suggest that low Nigerian BP vaccination rates led to high rates of circulating BP, high individual BP exposure rates, high rates of latent immunization and mucosal BP immunocompetence, and therefore to lower SCBPC and AD rates. In contrast, high US vaccination rates led to low rates of circulating BP, low individual BP exposure rates, low rates of latent immunization and mucosal BP immunocompetence, and to higher SCBPC and AD rates. We propose that the increased risk for AD in African Americans compared to Nigerians, and more broadly, the higher risk of AD in more affluent societies (Hendrie et al., 2001; Ukraitseva, 2016; Mathers and Leonardi, 2000), reflects a difference in BP vaccination and SCBPC rates.

To be clear, the rising age-adjusted risk for AD in the US correlates with, but is not held to be directly caused by, currently available pertussis vaccines which decrease acute clinical BP risk, but do not induce protective mucosal immunity, preclude subclinical BP infection (Ward et al., 2005; Zhang et al., 2014; Warfel et al., 2014) or prevent transmission (Warfel et al., 2014; Long et al., 1990). Subclinical BP colonization (not pertussis vaccination or acute clinical BP) is proposed to directly increase AD risk.

3. Plausibility of nasopharyngeal *Bordetella pertussis* infection and AD

3.1. Subclinical nasopharyngeal *Bordetella pertussis* colonization is common

BP infection is not commonly reported in adults, rates of subclinical infection are underappreciated, and SCBPC has been documented in multiple populations and age groups. In a highly (99%) vaccinated region in China in 2011, during a period without a recent BP outbreak, 4.8% of 629 asymptomatic children screened polymerase chain reaction (PCR) positive for BP by nasopharyngeal swab (Zhang et al., 2014). In a German study of sudden unexpected deaths in infants conducted from 1995 to 1997, 5.3% of 441 infants in the control group were colonized with BP, as doc-

umented by nasopharyngeal swab PCR (Heininger et al., 2004). In 2015, a cross sectional study reported 7.1% of 70 asymptomatic Iranian healthcare workers were positive for BP by nasopharyngeal culture (Naeini et al., 2015). In a Dutch study, the annual incidence of BP infection in 3–79 year olds between 1995 and 1996, as determined by serologic assays, was 6.6%, while the annual incidence of notified cases was just 0.01% (de Melker et al., 2006), for a ratio of reported to total BP cases of 1 in 660. In a subsequent Dutch study quantifying serologic evidence of BP infection in 2006–2007, 9.3% of those age 9 years and older had significantly elevated levels of anti-pertussis toxin IgG, suggesting BP infection in the prior year (De Greeff et al., 2010). An Italian study found that serologic evidence of recent BP infection increased from 9.3% (95% CI 7.5–11.1%) in 1997 to 14.1% (95% CI 11.4–16.8%) in 2013 (Palazzo et al., 2016). In the US acellular BP vaccine trial completed in 1999, rates of undiagnosed BP infections for individuals age 15–65 years were estimated at 1–10 million cases per year, depending on case definition (Ward et al., 2005), in years when the Centers for Disease Control and Prevention (CDC) reported approximately 7000 US cases annually (<http://www.cdc.gov/pertussis/surv-reporting/cases-by-year.html>). The ratio of reported to undiagnosed BP cases, comparing data from the Ward study to concurrent CDC data, ranges from 1 in 140 to 1 in 1400. In summary, over the past two decades multiple studies from across the globe document SCBPC rates greatly in excess of reported BP infection rates, including evidence from highly vaccinated populations.

3.2. Viability of a nose to brain pathway

Given documentation of nasopharyngeal BP colonization in multiple populations, we examine the potential for SCBPC-secreted toxins to access the CNS. While a detailed understanding of the nose to brain pathway is still emerging, extensive evidence supports its viability (Ferreyra-Moyano and Barragan, 1989; Malerba et al., 2011; Illum, 2004; Doty, 2008; Landis et al., 2012), and multiple prior authors have suggested that pathogens access the CNS via an olfactory route (Merril, 2012; Ferreyra-Moyano and Barragan, 1989; Doty, 2008; Gay, 2007) and may lead to neurodegenerative disease (Merril, 2012; Ferreyra-Moyano and Barragan, 1989; Doty, 2008). For purposes of this paper, the “nose” is meant to include the nasal cavity, nasopharynx and proximate structures with ciliated mucosal surfaces, including the paranasal sinuses.

BP is primarily transmitted from person to person by expelled airborne droplets from an infected individual (Broder et al., 2006), and once inhaled, can bind to mucosal ciliated respiratory epithelium that lines much of the respiratory tract (Dahl and Mygind, 1998). In addition, the nasal turbinates direct a portion of inspired air to ciliated olfactory epithelium of the superior nasal cavity (Elad et al., 1993), to which BP readily adheres (Mattoo and Cherry, 2005). With advanced age, respiratory epithelium can replace olfactory neuroepithelium at the superior nasal mucosa providing additional surface area for BP attachment adjacent to olfactory nerves, and this transition may occur more rapidly in those with AD (Hawkes, 2003; Yamagishi et al., 1994). Sensory olfactory nerves are also ciliated (Jenkins et al., 2009), providing an additional adhesive foothold for BP. As olfactory nerves pass through channels in the bony cribriform plate to terminate in the forebrain at the olfactory bulb (Jansson, 2004), they provide direct communication from the nasal cavity to the CNS and a path of travel for BP toxins secreted by an SCBPC infection in a mucosally BP-immunodeficient host.

Multiple intranasally administered drugs travel rapidly to the olfactory bulb, and the brief transit interval (5–90 min) suggests extracellular transport (Chapman et al., 2013). Mammalian studies demonstrate the passage of neuropeptides from the nose to brain without entering the bloodstream (Illum, 2000; Born et al., 2002), and Balin et al. established a direct extracellular pathway from

nose to brain by observing that, in mammalian models, intranasal horseradish peroxidase reaches the olfactory bulbs of the CNS within 45–90 min (Balin et al., 1986).

In addition to migration along the path of olfactory nerves, it is possible that BP toxin reaches the CNS *via* absorption from the nasal mucosa into nasal capillaries and circulation, eventually traversing the circumventricular organs (CVOs). CVOs are midline periventricular brain tissues, including the choroid plexus, median eminence, neurohypophysis, subfornical organ, organ vasculosum or lateral terminalis, area postrema, and the pineal gland (Johnson and Gross, 1993; Schulz and Engelhardt, 2005). These tissues lack the usual tight endothelial junctions of the blood-brain barrier (BBB), which allow for the physiologic passage of pituitary neuroendocrine hormones (Fry and Ferguson, 2007), while maintaining a more permeable boundary between the peripheral circulation and the CNS. Broadwell and Sofroniew have shown that serum proteins with mass of up to 500 kilodaltons (kDa) may access the CNS and cerebrospinal fluid (CSF) *via* CVOs (Broadwell and Sofroniew, 1993). By comparison, the mass of BP toxin is 105 kDa (Locht et al., 2011), well within size parameters for CVO-mediated passage into the CNS, though solubility and other factors may also play a role.

While migration of BP toxin into the CNS may not require facilitation beyond passage *via* olfactory nerve channels and CVOs, we note that BP toxin also impairs BBB integrity (Kügler et al., 2007; Kerfoot et al., 2004), which is compromised in AD (Ujiie et al., 2003).

3.3. The neuroanatomic link between olfactory dysfunction, nasopharyngeal SCBPC, and AD

Impaired olfactory detection and identification are common in AD, and olfactory dysfunction predicts progression from mild cognitive impairment to AD, and increases with AD severity (Arnold et al., 2010; Attems et al., 2005; Devanand et al., 2000; Roberts et al., 2015). Anosmia confers twice the risk of cognitive loss compared with those with normal olfactory function over just two years of follow up, and five times the cognitive risk in those with a single APOE ϵ 4 allele (Graves et al., 1999).

Deficits in odor detection may be due to olfactory sensory pathology observed at mucosal surfaces in AD (Yamagishi et al., 1994; Touhara, 2002; Bruch and Kalinoski, 1987), and postmortem human studies demonstrate increased levels of A β and paired helical filament-tau in the olfactory epithelium of AD patients compared with controls. In addition, preliminary data demonstrate higher levels of tau protein, including phosphorylated tau, in nasal secretions of anosmic AD patients compared with controls (Passali et al., 2015), consistent with the presence of peripheral (intranasal) olfactory pathology in AD. Notably, co-administration of tau protein and adjuvant including BP toxin produces CNS neurofibrillary tangles, axonal damage, and gliosis consistent with AD (Rosenmann et al., 2006) (see Section 4.3). Further, since part of odor discrimination is mediated by BP toxin sensitive G-protein coupled olfactory receptors (Touhara, 2002; Bruch and Kalinoski, 1987), e.g. G $_{\alpha i}$, components of odor signal transduction may be inhibited by secreted BP toxin (Ignatious Raja et al., 2014; Von Dannecker et al., 2005), contributing to olfactory deficits in AD.

As illustrated in Fig. 1, the anatomic location of SCBPC in the superior aspect of the nasal cavity, just inferior to the cribriform plate, is contiguous with neurologic projections into the CNS, paralleling the observed distribution of AD pathology. Affected proximal structures in AD include the olfactory epithelium, olfactory tract, olfactory bulb, primary olfactory cortices and their projections (Attems et al., 2014). Notably, a large autopsy study documented the correlation between Braak stage in AD and the degree of olfactory bulb deposition of A β , neuritic plaque and α -synuclein ($p < 0.001$) (Attems et al., 2014). Observed deficits in odor identification, and potentially detection, may also be due to more central

olfactory neuropathology and cognitive changes in AD (Serby et al., 1991; Nordin and Murphy, 1996; Hawkes and Shephard, 1998; Sakuma et al., 1996).

Primates retain direct projections from the olfactory tract to the hippocampus and amygdala, which not only suggests an important relationship between the olfactory and limbic systems (Zald and Pardo, 1997), but also provides an anatomic route for the transport of BP toxin from an SCBPC within the nasal cavity to these brain regions. As discussed in detail in Section 4 below, there are multiple mechanisms by which BP toxin may promote neuropathology consistent with AD. Ferreyra-Moyano and Barragan argued that since classic AD pathology in autopsied AD brains preferentially involves the olfactory and entorhinal cortices, the amygdala and the hippocampus, and because each of these brain regions receives primary or secondary projections from the olfactory bulb, the olfactory system likely plays a role in the pathogenesis of AD (Ferreyra-Moyano and Barragan, 1989).

Braak et al. classified AD into neuropathological stages, with pathologic progression over time and space, wherein cortical disease is first noted in the transentorhinal region of the temporal lobe (Braak and Braak, 1996; Braak and Del Tredici, 2013; Braak et al., 2011). Histologic mapping demonstrates that olfactory bulb pathology occurs early in AD (Braak stage 0 or 1, of 6 stages) (Hyman et al., 1990; Loopuijt and Sebens, 1990). A study of 40 definitive AD cases detailed tau pathology in the olfactory system, while another series used tau immunohistochemistry to document olfactory AD pathology (Attems et al., 2005; Kovacs et al., 1999). As Kovacs et al. summarized, in AD “The most severely affected areas are interconnected with the central olfactory system, in contrast with the relative sparing of other sensory areas without olfactory connections” (Kovacs et al., 1999).

Dystrophic olfactory neurites (Arnold et al., 2010) and cerebral atrophy are also common in AD, with hippocampal loss more than 4 times greater in AD than in controls (Leung et al., 2013). Characteristic progressive cerebral atrophy of AD positively correlates with Braak stage, with early regional brain changes in AD projecting from the olfactory tract (e.g. entorhinal cortex, anterior hippocampus), then spreading to the parietal lobes, temporoparietal association cortices, and the frontal lobes (Whitwell et al., 2007). Importantly, in the pre-vaccine era when deaths due to pertussis were high, Litvak et al. observed that cerebral atrophy was the most common cerebral complication of BP (Litvak et al., 1948), and Ford demonstrated that post-pertussis CNS neuropathology included cerebral cortical neurodegeneration, most notably in the hippocampus and frontal cortex (Ford, 1929), consistent with AD.

Beyond regional cerebral pathology, BP-mediated injury to even a single cell in the olfactory tract might lead to a “tip-ping the first domino” effect with pathologic consequences to neurally connected limbic structures, such as the hippocampus and amygdala. As previously summarized by Hardy and Revesz, AD may progress as a result of “spreading of disease—for example, between adjacent neurons in a pathway” (Hardy and Revesz, 2012). Liu et al. used mice transfected to express human tau to demonstrate sequential propagation of tau pathology from the entorhinal cortex along anatomically connected neural networks with apparent trans-synaptic spread (Liu et al., 2012). BP toxin localized to a single neuron leading to constitutive cyclic adenosine monophosphate (cAMP) production (*via* G $_i$ inhibition), protein kinase A (PKA) activation, and the subsequent upregulated phosphorylation of multiple substrates (e.g. tau, G-protein-coupled receptor kinase 2) is an example of such a potential BP-mediated first domino scenario (see Sections 4.4 and 4.14).

Others have shown that A β production can be increased in mice transfected to overexpress human amyloid precursor protein, through the introduction of A β “seeds” into the CNS, with

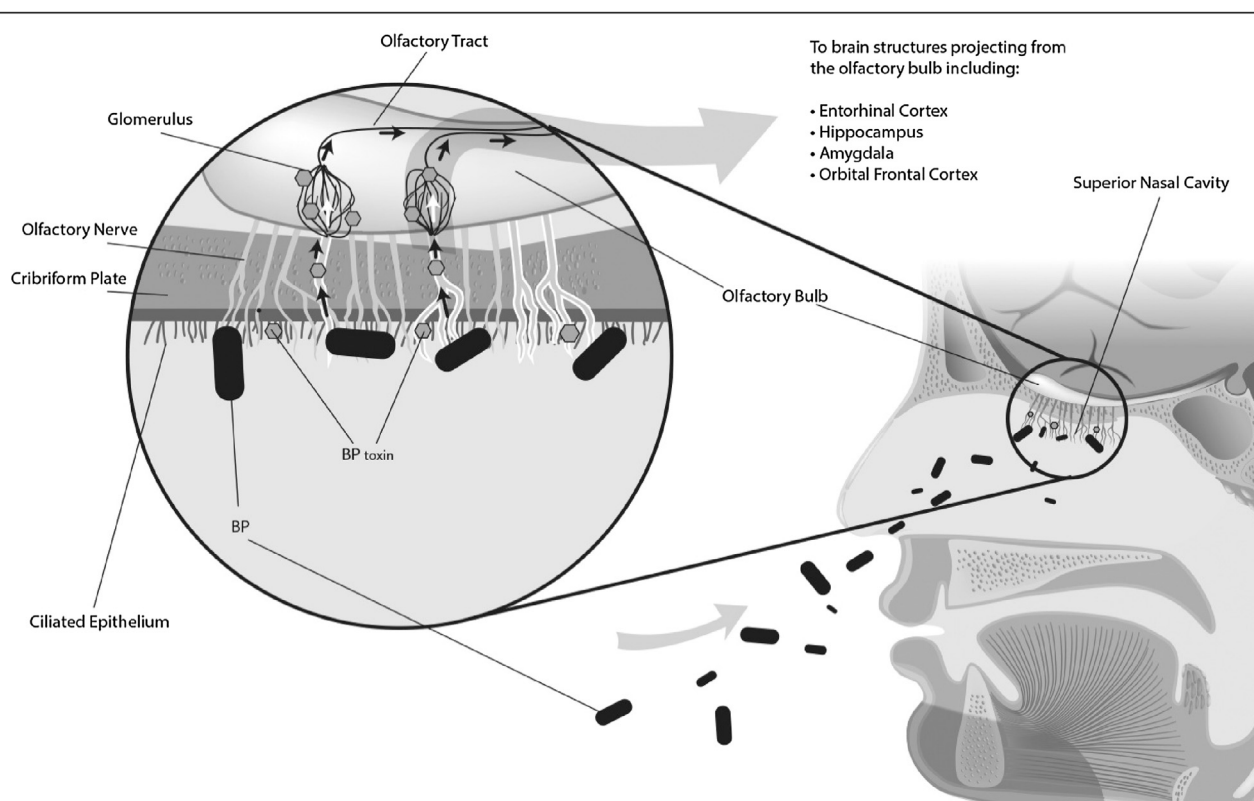


Fig. 1. Sagittal illustration of the human head demonstrating inhalation of *Bordetella pertussis* and localization to the superior nasal cavity. Note the close anatomic relationship between the natural habitat of BP colonization at the ciliated epithelium of the superior nasal cavity, and the adjacent olfactory nerves that pass through channels in the bony cribriform plate to enter the CNS at the olfactory bulb. We propose that BP toxin secreted by SCBPC in the superior nasal cavity can ascend along the olfactory tract (arrows), through channels in the cribriform plate, and localize to the olfactory bulb and to brain structures projecting from the olfactory bulb such as the entorhinal cortex, hippocampus and amygdala.

patterns of A β deposition approximating those observed in normal age-related deposition and AD, e.g. favoring the hippocampus (Eisele et al., 2009). As discussed in detail in Sections 4.1, 4.5 and 4.6, BP toxin may initiate and perpetuate A β accumulation to promote neuropathology characteristic of AD, given BP toxin's ability to induce A β (McManus et al., 2014) and A β -generating BACE1 (Hartlage-Rübsamen et al., 2003), and reduce A β clearance (Tahara et al., 2006; Chen et al., 2006). These findings suggest that small amounts of A β and abnormal tau, triggered by BP toxin, might initiate a cascade of protein deposition contributing to AD pathology, with propagation analogous to that seen in prion disease (Jucker and Walker, 2011; Guo et al., 2006; Tatarnikova et al., 2015).

In summary, AD pathology may sequentially progress through the CNS, starting with peripheral olfactory structures, tracking along neuroanatomic networks to the transentorhinal cortex and entorhinal cortex, the hippocampus and temporal neocortex and to other brain regions—a scenario consistent with nasally initiated, SCBPC-mediated, neuropathology. Given the proximity of the CNS to BP-hospitable mucosal surfaces of the superior nasal cavity, the contiguity of these ciliated epithelial BP binding sites and olfactory nerves which pass through the cribriform plate into the forebrain, the unusual ability of BP toxin to compromise the BBB, and the documented association between acute clinical BP and AD-stereotypic neuropathology, it is possible, if not probable, that BP toxin secreted by SCBPC infections accesses the CNS in some infected individuals. Potential pathology due to BP toxin activity within the CNS is discussed below in light of experimentally induced BP-mediated disease that is highly consistent with the hallmark pathologies of AD.

4. Mechanistic evidence supporting BP infection in AD

We review fifteen mechanisms by which BP infection may promote the development of AD. Among the evidence, BP or BP toxin (1) increases production and decreases clearance of A β fibrils, which comprise neuritic plaques, (2) increases production of hyperphosphorylated tau and neurofibrillary tangles, and (3) promotes CNS inflammation and neural excitotoxicity—neuropathology highly characteristic of AD. In addition, we note the deleterious interaction between infection and carriage of the APOE ϵ 4 allele, provide insight into mechanisms by which BP can induce mitochondrial dysfunction and oxidative stress, lead to AD as so-called “type 3 diabetes”, and disrupt the structure and function of the Golgi apparatus and endoplasmic reticulum to cause pathologic protein aggregation as observed in AD.

4.1. Respiratory infection with BP induces hallmark AD pathology

Perhaps the most striking evidence for the potential role of BP in AD are the important experiments conducted by McManus et al. demonstrating that respiratory (inhaled) infection with BP in APP/PS1 mice, transgenic for enhanced CNS human amyloid precursor protein production, significantly increases A β -containing plaques in the hippocampus and frontal cortex (McManus et al., 2014), recapitulating neuropathology typical of AD. In addition, these experiments establish that respiratory BP infection induces other AD-typical findings including CD4 $^{+}$ T helper 1 (Th1) lymphocyte and CD4 $^{+}$ T helper 17 (Th17) lymphocyte brain infiltration, and increased cortical expression of CD11b messenger ribonu-

cleic acid (mRNA) and glial fibrillary acidic protein mRNA—markers of CNS microglial activation and astroglial activation respectively (Kalla et al., 2003; Webster et al., 2006). These changes increase with mouse age and time since BP exposure (progression occurs even after infection clears), with McManus et al. suggesting, “infection may be a major environmental factor in the progression of AD-like pathology” (McManus et al., 2014). Considering the totality of evidence presented herein, we go further to propose that more specifically, *Bordetella pertussis* is an important human neuropathogen and that subclinical BP colonization (SCBPC) is a previously unidentified major cause of AD. We submit that AD neuropathology may be initiated by nasopharyngeal SCBPC, with local secretion of BP toxin. As noted in Section 2.3, SCBPC is more common among individuals in highly BP vaccinated populations with low circulating levels of BP. Such individuals possess poor mucosal BP immunity due to reduced BP exposure and thus reduced latent BP immunization, compounded by the inability of currently available BP vaccines to induce potent and effective mucosal immunity (Zhang et al., 2014; Warfel et al., 2014). Poor mucosal BP immunity, in turn, is held to increase the risk of SCBPC infection, and thus AD.

4.2. Respiratory BP infection induces CNS inflammatory changes consistent with AD

In addition to activating CNS microglia and astroglia (McManus et al., 2014; Kalla et al., 2003; Webster et al., 2006), respiratory BP infection can upregulate the inflammatory cytokines interleukin 1 β (IL-1 β) and tumor necrosis factor- α (TNF- α) in the hippocampus (Loscher et al., 2000), a brain region characteristically affected in AD. Importantly, “...TNF- α and IL-1 β increase expression of APP [A β PP] and A β , suggesting that CNS inflammation may in fact amplify amyloid deposition” (Holmes and Cotterell, 2009). Even at picomolar concentrations, BP toxin inhibition of G $_i$ proteins can directly lead to reduced microglial branching, a hallmark of the microglial activation and neuroinflammation observed in AD (Kalla et al., 2003; Griffin, 2013).

Experimental models also demonstrate that respiratory BP infection upregulates the pro-inflammatory cytokine interleukin 6 (IL-6) in the hippocampus and hypothalamus (Loscher et al., 2000), consistent with elevated cerebrospinal fluid IL-6 levels in AD patients (Blum-Degen et al., 1995).

4.3. Co-administration of tau and BP toxin produces neurofibrillary tangles

Mice injected with recombinant human tau protein and adjuvant including BP toxin develop CNS neurofibrillary tangles, axonal damage, and gliosis consistent with AD pathology (Rosenmann et al., 2006). Just as AD patients produce higher levels of anti-tau antibodies compared to patients with non-AD dementia and non-demented controls (Bartos et al., 2012), all mice immunized to tau in this study developed anti-tau antibodies (Rosenmann et al., 2006).

Given the presence of tau protein in the olfactory epithelium of elderly human adults without AD (Arnold et al., 2010; Trojanowski et al., 1991), and in nasal secretions of anosmic adults without AD (Passali et al., 2015), and given the significant prevalence of SCBPC, it is anticipated that tau, co-localized with BP toxin secreted from an SCBPC, manifests the neurofibrillary tangles, axonal damage and gliosis produced in experimental models (Rosenmann et al., 2006), contributing to neuropathology in AD.

4.4. BP toxin-mediated cAMP-dependent PKA dysregulation is consistent with tau hyperphosphorylation

BP toxin induces constitutive production of cAMP (Katada, 1982) which activates PKA (Seino and Shibasaki, 2005), which in turn

phosphorylates tau (Scott et al., 1993). Thus, BP toxin-mediated constitutive production of cAMP can lead to hyperphosphorylation of PKA substrates, including tau. Tau hyperphosphorylation by PKA induces the paired helical filaments of NFTs (Zheng-Fischhöfer et al., 1998). Hyperphosphorylated tau is a hallmark of AD and is thought to lead to the formation of insoluble tau, microtubule instability and abnormal aggregation of tau isoforms (Anderson et al., 2009; Schneider et al., 2004; Buee et al., 2000) commonly observed in AD (Zheng-Fischhöfer et al., 1998). As one group noted, “...the clinical progression of Alzheimer’s disease correlates with distribution and amount of tau aggregates...[and] A common hypothesis holds that tau hyperphosphorylation and subsequent detachment increases the pool of unbound tau beyond a critical concentration, thereby initiating its aggregation into paired helical filaments (gain of toxic function)” (Schneider et al., 2004).

In addition, tau dephosphorylation by endogenous phosphatases may be inhibited by BP toxin, given that BP toxin inhibits certain protein phosphatases (Buee et al., 2000; Chen et al., 1998).

4.5. BP induces β -site A β PP-cleaving enzyme, BACE1

BACE1 is produced by neurons and astrocytes and is required for the generation of A β (Tanzi and Bertram, 2005). BP induces astrocyte BACE1 expression in a murine model using systemically administered adjuvant including BP to activate glial cells in mice transgenic for the human AD-associated A β PP mutant (K670N-M671L) gene from a family with early-onset AD (Hartlage-Rübsamen et al., 2003). In this study, astrocytes induced to produce BACE1 were noted “in close proximity” to CNS A β plaques, suggesting that BP toxin localized to astrocytes may induce BACE1 and thereby lead to A β plaque formation.

4.6. BP toxin reduces A β clearance

BP toxin dramatically reduces microglial clearance of fibrillar A β *in vivo* in a murine AD model mutant for toll-like receptor 4, a receptor linked to microglial activation and A β clearance (Tahara et al., 2006). In addition, toll-like receptor 2-activated microglial internalization of A β is inhibited by BP toxin (Chen et al., 2006).

4.7. The APOE ϵ 4 allele may alter inflammatory responses to infection and favor the development of AD

APOE allelic variation may contribute to AD pathology through numerous mechanisms (Bu, 2009). Prior investigators have proposed that neurotropic infection is a risk factor for AD in those who carry the APOE ϵ 4 allele (Itzhaki et al., 2004) and “...the risk of developing AD is increased by infection or general ill health, and a particular susceptibility to infection may be conferred by the ApoE ϵ 4 allele” (McManus et al., 2014). Animal data also suggest that presence of the APOE ϵ 4 allele confers increased permeability of the blood-brain barrier (Nishitsuji et al., 2011), which may increase CNS susceptibility to penetration by BP toxin.

Animal data support immunologic and inflammatory responses to bacterial toxins which promote AD neuropathology in carriers of the APOE ϵ 4 allele (Zhang et al., 2010). For example, in experimental autoimmune encephalomyelitis using BP toxin as an adjuvant, APOE ϵ 4-transgenic mice demonstrate AD-stereotypic tasks, such as deficits in spatial learning and recall (Tu et al., 2009). In this model, choline acetyltransferase is reduced in the hippocampus (Tu et al., 2009), just as decreases in hippocampal choline acetyltransferase activity correlate with cognitive loss in AD (Pappas et al., 2000).

4.8. BP toxin increases excitotoxic neural glutamate release

Glutamate is the major excitatory CNS neurotransmitter in mammals where it mediates fast synaptic transmission, and excitotoxic cell death results from prolonged depolarization of neurons, as seen in AD (Doble, 1999; Hynd et al., 2004). Glutamate release from CNS neurons includes cAMP and PKA dependent mechanisms (Herrero and Sánchez-Prieto, 1996), levels of which can be increased through $G_{i/o}$ inhibition of glutamate receptors sensitive to BP toxin (Katada, 1982; Padgett and Slesinger, 2010). BP toxin increases neuronal glutamate release (Cullen et al., 1994; Huston et al., 1993) and converts adenosine induced inhibition of neural glutamate release to stimulation of neural glutamate release (Dolphin and Prestwich, 1985). Compounding this excitotoxic increase in glutamate release is the ability of BP toxin to inhibit $G_{i/o}$ gamma-aminobutyric acid B (GABA_B) receptors, which mediate certain neuroinhibitory functions in the CNS (Padgett and Slesinger, 2010). In sum, it is plausible that BP toxin increases CNS glutamate-mediated excitotoxicity while decreasing GABA_B neuroinhibition, promoting excitotoxicity as seen in AD, essentially “depressing the accelerator while releasing the break.”

4.9. BP toxin inhibits secretion of brain-derived neurotrophic factor (BDNF)

BDNF supports the maintenance and repair of neurons and is expressed in multiple brain regions, including the hippocampus (Binder and Scharfman, 2004) and olfactory bulb (Mast and Fadool, 2012), areas preferentially affected in AD (Attems et al., 2014; Braak et al., 2011). BDNF is neuroprotective against A β *in vivo* (Arancibia et al., 2008). Function-blocking anti-BDNF antibodies impair both short-term and long-term memory formation in mice (Alonso et al., 2002), such that impaired BDNF activity replicates memory deficits seen in AD. Notably, BDNF levels are depressed in AD (Henry et al., 2010).

Consistent with these observations, BP toxin inhibits the secretion of BDNF (Gunther et al., 2000) from CNS neurons *in vivo*, and may also inhibit calcitonin gene-related peptide (CGRP) enhanced BDNF release, as the CGRP receptor is BP toxin sensitive (Buldyrev et al., 2006). If BP toxin were to access the hippocampus or other areas of the brain that rely on BDNF for neurotrophic support, it is plausible that BP toxin-induced decreased BDNF activity would contribute to neuropathology in AD.

4.10. BP toxin inhibits Wnt signaling

For more than 15 years, Wnt signaling has been proposed to influence multiple pathologic mechanisms in AD: “...loss of function of Wnt signaling components would trigger a series of misrecognition events, determining the onset and development of AD” (De Ferrari and Inestrosa, 2000). For example, Wnt signaling regulates adult hippocampal neurogenesis (Lie et al., 2005), A β –induced neuroinflammation and neurotoxicity (Li et al., 2011), and β -catenin dependent and independent proinflammatory activation of microglia (Halleskog and Schulte, 2013). Since BP toxin blocks several forms of Wnt signaling (Halleskog and Schulte, 2013; Pon and Wong, 2007; Kilander et al., 2011), CNS localized BP toxin from an SCBPC may contribute to AD *via* compromise of Wnt pathways.

4.11. BP toxin inhibits neuronal processes underlying memory formation

BP toxin inhibits long-term potentiation in hippocampal neurons (Goh and Pennefather, 1989), a mechanism underlying learning and memory formation (Whitlock et al., 2006). In addition,

BP toxin reduces synaptic transmission and blocks long-term depression in corticostriatal synapses, a proposed mechanism in motor learning and memory (Tang and Lovinger, 2000). By these and other mechanisms discussed herein, BP toxin can impair memory, the signature pathology of AD.

4.12. BP toxin blocks neuroprotection by soluble amyloid precursor protein α (sAPP α)

Membrane bound sAPP α is neuroprotective *in vivo* in murine hippocampal cells, acting *via* the Akt survival pathway and likely through inhibition of glycogen synthase kinase 3 β (GSK3 β)-induced apoptosis (Milosch et al., 2014). Akt activation is mediated by signal transducing G proteins sensitive to BP toxin, administration of which abolishes sAPP α -mediated cell survival (Milosch et al., 2014). In sum, BP toxin inhibits a neural rescue pathway, which may exacerbate apoptotic cell loss in AD.

4.13. BP induces mitochondrial dysfunction and oxidative stress

Mitochondrial dysfunction and oxidative stress have been implicated in the early pathophysiology of AD, including neuronal pathology induced by reactive oxygen species (Hirai et al., 2001; Blass and Gibson, 1990; Su et al., 2008). Consistent with these findings, BP alters cellular cAMP levels, mitochondrial membrane potentials and adenosine triphosphate (ATP) levels, and can thereby lead to BP induced apoptosis (Bachelet et al., 2002). In addition, BP toxin possesses nicotinamide adenine dinucleotide (NAD⁺) glycohydrolase activity (Locht et al., 2011; Kaslow and Burns, 1992) which can deplete cytosolic NAD⁺ (a coenzyme in redox reactions) and thereby lead to glycolytic failure, mitochondrial membrane depolarization and altered permeability which can stimulate apoptosis-inducing factor release, and neuronal death (Alano et al., 2010). Since NAD⁺ levels decrease in degenerating neurons and preserved axonal NAD⁺ levels protect against neurodegeneration (Wang et al., 2005), BP-mediated depletion of cellular NAD⁺ may contribute to AD neurodegeneration. BP also induces leukocyte production of hydrogen peroxide (Steed et al., 1992), which decomposes to form hydroxyl radicals that can lead to oxidative damage (Barber et al., 2006). Further, in the experimental autoimmune encephalomyelitis model, BP toxin contributes to the induction of subclinical neuronal dysfunction which correlates with oxidative stress, as indicated by over-activation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidases (Radbruch et al., 2016).

4.14. BP toxin inhibits CNS insulin receptor activation, abolishes glucose transporter 4 (GLUT4) translocation, and increases serum insulin levels

Glucose uptake and utilization is critical to brain function and is mediated by glucose transporter (GLUT) proteins, which are regulated by insulin and highly expressed in medial temporal brain structures, notable sites of AD pathology (de la Monte, 2014). GLUT4 is expressed in the olfactory bulb, hippocampus, and cortex (Benomar et al., 2006) and brain insulin resistance occurs both early and frequently in AD (Persiyantseva et al., 2013). The importance of CNS insulin resistance in neurodegeneration and impaired brain glucose utilization has led some to refer to AD as “Type 3 Diabetes” (Steen et al., 2005).

In CNS neurons, BP toxin inhibits insulin-stimulated receptor autophosphorylation, a critical step in insulin receptor activation (Persiyantseva et al., 2013). In addition, since $G_{\alpha i2}$ regulates translocation of GLUT4 from the cytosol to the plasma membrane, and since BP toxin catalyzes ADP-ribosylation and inactivates $G_{\alpha i2}$, BP toxin abolishes GLUT4 translocation and thereby impairs glucose

transport (Song et al., 2001). Disruption of GLUT4 translocation in the hippocampus is associated with memory impairment (Winocur et al., 2005).

Further disruption of CNS glucose uptake may occur from BP toxin-mediated constitutive activation of cAMP production (Katada, 1982) which, as previously noted (see Section 4.4), regulates PKA signaling (Seino and Shibasaki, 2005). PKA increases phosphorylation of G-protein-coupled receptor kinase 2 (GRK2) and enhances recruitment of GRK2 to the plasma membrane (Li et al., 2006). GRK2 upregulation is observed in AD (Leosco et al., 2007) and increased GRK2 inhibits both insulin-stimulated GLUT4 translocation and uptake of 2-deoxyglucose (Usui et al., 2004), providing an additional potential BP-mediated mechanism to account for the insulin resistance (Craft, 2007) and compromised cerebral glucose metabolism and uptake observed in AD patients (Liu et al., 2008). It is noteworthy that in AD brains, reductions in glucose transporter proteins correlate with abnormal hyperphosphorylation of tau (Liu et al., 2008), another PKA substrate (see Section 4.4).

Finally, hyperinsulinemia is associated with higher AD risk and decline in memory-related cognitive function (Luchsinger et al., 2004). In rats, hyperinsulinemia leads to “defects in hippocampal synaptic plasticity associated with deterioration in spatial learning and memory functions” (Kamal et al., 2013). In a human randomized crossover trial, moderate hyperinsulinemia induction in healthy adults led to increased CNS cytokine levels, including IL-1 β , IL-6, and TNF- α , “...agents that interact synergistically to promote A β synthesis (IL-6 and IL-1 β) and reduce its clearance (TNF- α).” (Fishel et al., 2005)

Consistent with these observations, BP toxin administered to animals and healthy human subjects, even as a single dose, leads to increased serum insulin levels with “a long duration of action” (Toyota et al., 1980; Yajima et al., 1983). Further, it has been demonstrated that humans infected with BP can present with increased serum insulin levels without hypoglycemia (Furman et al., 1988). Of historical interest, BP toxin was formerly known as Islet Activating Protein for its ability to stimulate pancreatic insulin release and raise serum insulin levels.

4.15. BP toxin disrupts the structure and function of the Golgi apparatus and endoplasmic reticulum which can lead to protein aggregation

Characteristic AD histopathology includes CNS protein accumulation with aggregation of both tau and A β , and it has been suggested that “AD results from protein aggregation... [which] represents a key starter and/or promoter of the disease” (Thal and Fändrich, 2015).

The Golgi Apparatus (GA) is the central cellular organelle in the secretory pathway, processing and targeting proteins for distribution throughout the cell (Nassif et al., 2010). The contribution of GA fragmentation and dysfunction to the pathogenesis of neurodegenerative disease including AD has been previously reviewed (Nassif et al., 2010; Fan et al., 2008; Gonatas et al., 2006; Nakagomi et al., 2008).

GA size is a reliable index of neuronal activity and postmortem examination of AD brains demonstrates GA atrophy and fragmentation with neuronal atrophy in neurons without NFTs (Stieber et al., 1996). In neurons from the hippocampus, cerebellum, and acoustic and visual cortices of AD brains, there are fewer vacuoles and vesicles associated with the GA (Baloyannis, 2015). As previously summarized, “The morphological alterations of GA, which are observed even at the initial stages of AD, ... favor... the hypothesis that impairment of trafficking in Golgi cisternae and endosomes and ER stress may be one of the crucial factors in amyloidogenesis” (Baloyannis, 2015). Others have “hypothesize[d] that Golgi

fragmentation in AD accelerates APP [A β PP] trafficking and A β production” (Joshi and Wang, 2015).

Consistent with the disruption of GA structure and function observed in AD, BP toxin localizes to the GA (Plaut and Carbonetti, 2008), ADP-ribosylates G α_{i-2} proteins associated with GA (Kugler et al., 2007), and disrupts the structural integrity of the GA (Kugler et al., 2007; Hidalgo et al., 1995). Hidalgo et al. determined that treating cells with BP toxin stimulates GA disassembly and redistribution (Hidalgo et al., 1995). Kugler et al. observed that “In [brain microvascular endothelial] cells incubated with PT [BP toxin] for up to 6 h [hours], a progressive dissociation of Golgi structures was apparent” (Kugler et al., 2007). In short, BP toxin localizes to the GA and can lead to GA fragmentation that may thereby initiate and accelerate AD-typical pathology, including aberrant A β PP trafficking and production of A β .

The endoplasmic reticulum (ER) plays an important role in post-translational modification and maintenance of intracellular calcium homeostasis, and ER dysfunction and ER stress have been reported in animal models of AD and postmortem human AD brains (Li et al., 2015). Intraneuronal A β accumulation associated with ER stress is thought to be an early event in AD, and evidence suggests “a close linkage between ER-stress and tau pathology” (Li et al., 2015).

Consistent with the disruption of ER structure and function observed in AD, BP toxin localizes to the ER (Plaut and Carbonetti, 2008) and causes the “redistribution and fragmentation of the ER”, with loss of “regular parallel arrangement of sheets of rough ER” and reduced density of smooth ER compared to normal controls (Wang et al., 2000). Further, the G protein, G $_i$, also regulates GA-ER retrograde transport such that the inhibition of G $_i$ proteins by BP toxin stimulates GA redistribution into the ER (Hidalgo et al., 1995). Given the potential role of GA-ER retrograde transport in the processing and “quality control” of misfolded proteins (Hidalgo et al., 1995; Hammond and Helenius, 1994), dysregulation of this pathway by BP toxin may contribute to the characteristic pathologic accumulation of misfolded proteins seen in AD (DeToma et al., 2012).

As a site for calcium storage, the ER also plays a role in calcium homeostasis (Rao et al., 2002) and abnormal calcium homeostasis can lead to ER stress (Nassif et al., 2010). BP toxin affects activation of store-operated calcium channels as represented by inhibition of vasopressin-stimulated calcium inflow (Wang et al., 2000). BP toxin inhibits thapsigargin-induced calcium inflow without impairing calcium release from intracellular stores (Fernando and Barritt, 1994) and also inhibits calcium current depression by somatostatin and muscarinic agonists (Beech et al., 1992). By these and other mechanisms, BP toxin can alter cellular calcium homeostasis, dysregulating the ER and thus potentially furthering neuropathology in AD.

Taken together, ER and Golgi dysfunction may not be the result of toxic protein aggregates such as A β peptides as previously proposed (Ferreiro et al., 2006; Hitomi et al., 2004). Instead, BP-mediated disruption of the structure and function of GA and ER may be primary initiating neuropathogenic events leading to intracellular accumulation of misfolded and toxic protein aggregates, the aberrant release of extracellular proteins, and neurodegeneration.

Notably, in the presenilin (PS) gene mutations that predispose to familial AD, ER damage and loss of ER function are observed, and PS1 mutations further disrupt intracellular calcium homeostasis and sensitize neurons to cell death by promoting ER-mediated apoptotic proteolysis (Li et al., 2015; Zatti et al., 2006). Combined with the ability of BP to upregulate BACE1 in the familial early-onset human AD-associated A β PP mutant model (see Section 4.5), these observations provide potential mechanistic links between the BP-mediated sporadic AD pathology proposed in this paper and inherited pathogenic mechanisms contributing to familial AD.

5. Assessing causation with the Bradford Hill guidelines

While contributions from both genetic predisposition and environmental exposures are evident in AD, we summarize evidence for a novel hypothesis and acquired cause of AD: subclinical *Bordetella pertussis* colonization (SCBPC). Better known to induce whooping cough, BP is largely unappreciated as a frequent colonizer of the human nasopharynx. Using case studies and experimental data, we employ the Bradford Hill criteria as a benchmark of causal validation to assess the relationship between BP and AD, including strength, consistency, temporality, biological gradient, plausibility, coherence, and analogy to causally accepted mechanisms (Hill, 1965). Hill's construct organizes our analysis of the evidence for BP in the etiology of AD.

The BP-AD association is strong, with a statistically significant relative risk of 0.4 (a 60% risk reduction) for the development of AD over 5 years in those over age 65 reporting prior vaccination for tetanus or diphtheria, and thus likely BP. The association between AD and SCBPC is consistent across populations and time, as seen in the US over the past two decades, and in both Canada and Nigeria in the 1990s. The association is temporally logical as vaccination that commonly includes BP antigens is correlated with a decreased subsequent risk of AD, and strikingly, respiratory infection with BP in animal models precedes the deposition of A β plaques in the hippocampus and frontal cortex, and leads to other neuropathology highly characteristic of AD. The association exhibits a biologic gradient, as seen with the rising age-adjusted risk for AD in the US since the early 1990s, coincident with the rise in BP, the only vaccine-preventable disease in the US increasing in incidence during this period. Causation of AD by BP is plausible and coherent given the cross-cultural documentation of nasopharyngeal SCBPC, its proximity to the CNS, multiple access routes from the nasal mucosa to the CNS, the preferential and sequential distribution of AD pathology in the olfactory pathway and its projections, and the many and diverse sources of mechanistic corroboration cited. This extensive experimental support for BP as a cause of AD includes the ability of respiratory BP infection to induce A β -containing plaques and increase CNS inflammation, for BP toxin to reduce clearance of A β , induce BACE1, produce neurofibrillary tangles in mice when co-administered with tau, and preferentially affect APOE ϵ 4 carriers to alter inflammatory responses to infection that favor the development of AD pathology. Further, the hypothesis that BP is a cause of AD parallels our understanding of other diseases that were not initially recognized as infectious in origin. For example, the role of *H. pylori* in peptic ulcer disease (Marshall and Warren, 1984) is analogous to BP in AD in that *H. pylori* also colonizes the mucous layer (in this case, of the stomach) (Shimizu et al., 1996), may adhere to epithelium (Logan, 1996), and causes disease through the release of enzymes (Mobley, 1996) and toxins (Suerbaum and Michetti, 2002), or by eliciting an immune response (Mobley, 1996; Ernst et al., 1994).

Finally, given that BP has previously been proposed by the authors to play a causal role in the etiology of multiple sclerosis (MS) (Rubin and Glazer, 2016), findings of overlapping pathology are anticipated and have been previously reported. These findings include increased hyperphosphorylated tau (Anderson et al., 2009; Bartosik-Psujek and Stelmasiak, 2006) and increased A β (Odinak et al., 2008) in the CNS of clinical MS cases compared to controls, hyperphosphorylation and aggregation of insoluble tau in the principle animal model of MS (Schneider et al., 2004; Anderson et al., 2008), "profound" microglial activation in the CNS of both AD and MS patients with "strikingly similar" activation patterns (Dal Bianco et al., 2008), and in 8 of 45 MS brains examined at autopsy "plaque and tangle density exceeded the number required for neuropathological diagnosis of probable AD" with hippocampal, temporal, and entorhinal cortical AD pathology trending greater than that

in age-matched controls age 65 years and older (Dal Bianco et al., 2008). Also supporting the overlap of MS and AD pathology are the presence of high levels of A β PP in actively demyelinating MS plaques (Gehrmann et al., 1995), that CD45+ lymphocytes and pro-inflammatory cytokines in the CSF of patients with mild cognitive impairment (a preclinical stage of AD) were "at levels similar" to MS patients (Monson et al., 2014), evidence of CNS demyelination in early and late onset AD (Fornari et al., 2012; Mitew et al., 2010), and the observation of "similar autobiographical memory impairment" in MS and AD (Müller et al., 2013).

6. Conclusion

A *Bordetella pertussis* based hypothesis for Alzheimer's disease accounts for several intriguing epidemiologic observations and explains all hallmark AD neuropathology. BP is naturally predisposed to cause AD in its ability to colonize the human nasopharynx, co-localize with olfactory nerves which access the CNS, and particularly for the potent and diverse biologic effects of its eponymous exotoxin, demonstrated by numerous investigators to induce CNS pathology consistent with AD. Specifically, respiratory BP infection and BP toxin secreted from subclinical BP colonization can account for the initiation and accumulation of A β plaques and tau tangles, microglial activation and inflammation, atrophy and neurodegeneration, excitotoxicity, distinctive anatomic distribution and sequential spread of disease, oxidative stress, impaired glucose utilization, and other characteristic CNS pathology.

Given the confluence of AD and BP-mediated pathology, the high cross-cultural prevalence of SCBPC, and the lack of preventable or treatable causes with high attributable risk, research into BP as an important and preventable cause of AD is warranted. Probative investigation includes screening for olfactory tract BP-PCR in mild cognitive impairment (MCI) with its attendant increased risk of progression to AD, SCBPC surveillance of cohorts followed for MCI and AD onset, and BP colonization eradication studies with MCI and AD as outcome measures. In light of the evidence presented, further inquiry into the role of subclinical *Bordetella pertussis* colonization in the etiology of Alzheimer's disease offers hope, and presents a promising new direction for the prevention and treatment of this emerging, potentially infection-driven, epidemic.

Conflict of interest

KR and SG are employed by and hold an equity interest in ILiAD Biotechnologies, which is developing a vaccine for the prevention of *Bordetella pertussis*.

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